



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2020

Structural and functional diversity calls for a new classification of ABC transporters

Thomas, Christoph ; Aller, Stephen G ; Beis, Konstantinos ; Carpenter, Elisabeth P ; et al ; Martinoia, Enrico

Abstract: Members of the ATP-binding cassette (ABC) transporter superfamily translocate a broad spectrum of chemically diverse substrates. While their eponymous ATP-binding cassette in the nucleotide-binding domains (NBDs) is highly conserved, their transmembrane domains (TMDs) forming the translocation pathway exhibit distinct folds and topologies, suggesting that during evolution the ancient motor domains were combined with different transmembrane mechanical systems to orchestrate a variety of cellular processes. In recent years, it has become increasingly evident that the distinct TMD folds are best suited to categorize the multitude of ABC transporters. We therefore propose a new ABC transporter classification that is based on structural homology in the TMDs.

DOI: <https://doi.org/10.1002/1873-3468.13935>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-196583>

Journal Article

Published Version


















The following work is licensed under a Creative Commons: Attribution 4.0 International (CC BY 4.0) License.

Originally published at:

Thomas, Christoph; Aller, Stephen G; Beis, Konstantinos; Carpenter, Elisabeth P; et al; Martinoia, Enrico (2020). Structural and functional diversity calls for a new classification of ABC transporters. FEBS letters, 594(23):3767-3775.

DOI: <https://doi.org/10.1002/1873-3468.13935>

Structural and functional diversity calls for a new classification of ABC transporters

Christoph Thomas¹ , Stephen G. Aller² , Konstantinos Beis^{3,4} , Elisabeth P. Carpenter⁵, Geoffrey Chang⁶, Lei Chen^{7,8}, Elie Dassa⁹, Michael Dean¹⁰ , Franck Duong Van Hoa¹¹, Damian Ekiert¹², Robert Ford¹³, Rachelle Gaudet¹⁴, Xin Gong¹⁵, I. Barry Holland¹⁶, Yihua Huang¹⁷, Daniel K. Kahne¹⁸, Hiroaki Kato¹⁹, Vassilis Koronakis²⁰, Christopher M. Koth²¹, Youngsook Lee²², Oded Lewinson²³, Roland Lill²⁴ , Enrico Martinoia^{25,26}, Satoshi Murakami²⁷ , Heather W. Pinkett²⁸ , Bert Poolman²⁹ , Daniel Rosenbaum³⁰, Balazs Sarkadi³¹, Lutz Schmitt³² , Erwin Schneider³³, Yigong Shi³⁴, Show-Ling Shyng³⁵, Dirk J. Slotboom²⁹ , Emad Tajkhorshid³⁶, D. Peter Tieleman³⁷ , Kazumitsu Ueda³⁸ , András Váradi³¹ , Po-Chao Wen³⁶ , Nieng Yan³⁹, Peng Zhang⁴⁰, Hongjin Zheng⁴¹, Jochen Zimmer⁴² and Robert Tampé¹ 

- 1 Institute of Biochemistry, Biocenter, Goethe University Frankfurt, Germany
- 2 Department of Pharmacology and Toxicology, University of Alabama at Birmingham, AL, USA
- 3 Department of Life Sciences, Imperial College London, London South Kensington, UK
- 4 Rutherford Appleton Laboratory, Research Complex at Harwell, Didcot, UK
- 5 Structural Genomics Consortium, University of Oxford, UK
- 6 Skaggs School of Pharmacy and Pharmaceutical Sciences and Department of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, CA, USA
- 7 State Key Laboratory of Membrane Biology, Institute of Molecular Medicine, Beijing Key Laboratory of Cardiometabolic Molecular Medicine, Peking University, Beijing, China
- 8 Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China
- 9 Institut Pasteur, Paris Cedex 15, France
- 10 Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Gaithersburg, MD, USA
- 11 Department of Biochemistry and Molecular Biology, Faculty of Medicine, Life Sciences Institute, University of British Columbia, Vancouver, BC, Canada
- 12 Department of Cell Biology and Department of Microbiology, New York University School of Medicine, NY, USA
- 13 Faculty of Biology, Medicine and Health, The University of Manchester, UK
- 14 Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA
- 15 Department of Biology, Southern University of Science and Technology, Shenzhen, China
- 16 Institute for Integrative Biology of the Cell (I2BC), Université Paris-Sud, Orsay, France
- 17 National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China
- 18 Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA
- 19 Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Japan
- 20 Department of Pathology, University of Cambridge, UK
- 21 Structural Biology, Genentech Inc., South San Francisco, CA, USA
- 22 Division of Integrative Bioscience and Biotechnology, POSTECH, Pohang, Korea
- 23 Department of Biochemistry, The Bruce and Ruth Rappaport Faculty of Medicine, The Technion-Israel Institute of Technology, Haifa, Israel
- 24 Institut für Zytobiologie, Philipps-Universität Marburg, Germany
- 25 Department of Plant and Microbial Biology, University Zurich, Switzerland
- 26 International Research Centre for Environmental Membrane Biology, Foshan University, Foshan, China
- 27 Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan
- 28 Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA
- 29 Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, The Netherlands
- 30 Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, TX, USA
- 31 Institute of Enzymology, Research Center for Natural Sciences (RCNS), Budapest, Hungary
- 32 Institute of Biochemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
- 33 Department of Biology/Microbial Physiology, Humboldt-University of Berlin, Germany
- 34 Institute of Biology, Westlake Institute for Advanced Study, School of Life Sciences, Westlake University, Hangzhou, China
- 35 Department of Chemical Physiology and Biochemistry, Oregon Health & Science University, Portland, OR, USA

Abbreviations

ABC, ATP-binding cassette; cryo-EM, cryogenic electron microscopy; NBD, nucleotide-binding domain; TMD, transmembrane domain.

- 36 Department of Biochemistry, Center for Biophysics and Quantitative Biology, NIH Center for Macromolecular Modeling and Bioinformatics, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, IL, USA
37 Department of Biological Sciences and Centre for Molecular Simulation, University of Calgary, AB, Canada
38 Institute for Integrated Cell-Material Sciences (WPI-iCeMS), KUIAS, Kyoto University, Japan
39 Department of Molecular Biology, Princeton University, NJ, USA
40 National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
41 Department of Biochemistry and Molecular Genetics, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA
42 Molecular Physiology and Biological Physics, University of Virginia School of Medicine, Charlottesville, VA, USA

Correspondence

C. Thomas and R. Tampé, Institute of Biochemistry, Biocenter, Goethe University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt/M., Germany
Tel: +49-(0)69-798-29468; +49-(0)69-798-29475
E-mails: c.thomas@em.uni-frankfurt.de (CT); tampe@em.uni-frankfurt.de (RT)

(Received 27 June 2020, revised 19 August 2020, accepted 8 September 2020, available online 26 October 2020)

doi:10.1002/1873-3468.13935

Edited by Gergely Szakács

Members of the ATP-binding cassette (ABC) transporter superfamily translocate a broad spectrum of chemically diverse substrates. While their eponymous ATP-binding cassette in the nucleotide-binding domains (NBDs) is highly conserved, their transmembrane domains (TMDs) forming the translocation pathway exhibit distinct folds and topologies, suggesting that during evolution the ancient motor domains were combined with different transmembrane mechanical systems to orchestrate a variety of cellular processes. In recent years, it has become increasingly evident that the distinct TMD folds are best suited to categorize the multitude of ABC transporters. We therefore propose a new ABC transporter classification that is based on structural homology in the TMDs.

Keywords: ABC transporters; ATPases; cryo-EM; membrane proteins; molecular machines; phylogeny; primary active transporters; sequence alignment; structural biology; X-ray crystallography

We suggest a new classification of the ABC transporter superfamily that is based on the TMD fold. Historically, first hints of the ABC protein superfamily came from sequence alignments of bacterial proteins that revealed highly conserved motifs in their ATPase domains [1]. The superfamily of ABC proteins was subsequently divided into three main classes [2–4]: exporters, nontransporter ABC proteins, and a third class consisting primarily of importers. The mammalian ABC systems, in particular, were grouped into seven subfamilies (ABCA to ABCG), based on NBD and TMD sequence homology, gene structure, and domain order [5–7]. It should be noted that ABCE and ABCF are not transporters, but exist as twin-NBDs without TMDs and are involved in mRNA translation control [8]. Detailed membrane topology and sequence analyses of exporters uncovered that, in contrast to the NBDs, the TMDs are polyphyletic and can serve as references to categorize ABC transporters into three distinct types (ABC1–3) [9,10]. According to this classification, the cystic fibrosis transmembrane

conductance regulator (CFTR), the transporter associated with antigen processing (TAP), and the drug efflux pump P-glycoprotein (P-gp) belong to the ABC1 transporters; ABCG2 and ABCG5/G8 are members of the ABC2 group, which also comprises importers; and the macrolide translocator MacB is categorized as an ABC3 system. Yet, another classification scheme currently in use differentiates between the three types of importers predominantly found in prokaryotes [11–14] and two types of exporters, exemplified by Sav1866 [15] and ABCG5/8 [16], in addition to the LptB₂FG-type [17,18] and MacB-type [19–22] transporters.

Our motivation for proposing a revised nomenclature stems from the recent wealth of ABC transporter structures determined by X-ray crystallography and single-particle cryo-electron microscopy, which has unveiled a remarkable diversity of TMD folds and evolutionary relationships between bacterial and eukaryotic/mammalian transporters [16–21,23–26]. This affluence of structural information provides the opportunity to introduce a universal nomenclature that

combines previous phylogenetic analyses with the new findings coming from high-resolution structures. The nomenclature groups ABC transporters into distinct types, I–VII, based on their TMD fold (Fig. 1, Tables 1 and 2). This classification is supported by quantitative analyses using TM-scores based on pairwise structural alignment of TMDs (Tables S1–S6, Fig. S1). The classification focuses on the transporter-forming TMDs and does not consider additional membrane integrated domains, as for example observed in TAP1/TAP2 [27,28].

As before, types I–III of the new nomenclature cover the three different importer architectures (Fig. 1, Table 1, Tables S2 and S3; TM-score for pairwise structural alignment between the type III systems CbiQ (PDB code 5X3X) and EcfT from *Lactobacillus brevis* (PDB code 4HUQ): 0.736). It is noteworthy that prokaryotic importers typically operate with periplasmic, extracellular, or membrane-embedded substrate-binding proteins whose structural features correlate with the type of TMD fold [29].

Based on the characteristic structure of the founding member Sav1866, which includes a domain-swapped TMD arrangement, type IV members of the new nomenclature have previously been classified as type I ABC exporters [15]. However, a significant and growing number of these ABC proteins have nonexporter functions, i.e., the gated chloride channel CFTR, the regulatory K_{ATP} channel modules SUR1/2, the lysosomal cobalamin (vitamin B₁₂) transporter ABCD4 [30], the bacterial siderophore importers YbtPQ and IrtAB, and the cobalamin/antimicrobial peptide importer Rv1819c [31–33], as well as several type IV systems with importer functions in plants [34–39]. This striking functional diversity mediated by the same structural framework (Fig. 1, Tables 1 and 2, Tables S4 and S5) makes the type IV ABC transporters stand out and is also the main reason why we suggest the more universal taxonomy based on structural principles.

According to the new classification, type V systems are ABC transporters of the ABCG/ABCA/Wzm type (Fig. 1, Tables 1 and 2, Table S6). They include channel-forming biopolymer secretion systems in bacteria [25,26]. Remarkably, although many type V systems are exporters, this type also comprises transporters with import function, including the retina-specific importer (flippase) ABCA4 (rim protein) [40,41] and importers in plants [42–44].

Finally, LptB₂FG and MacB are the founding members of type VI and type VII ABC transporters, respectively. We are aware that LptF and LptG have TMD folds that resemble type V members, and the TMD of MacB is reminiscent of type V systems and

LptF/G. Yet, they exhibit distinct features that warrant classifications into separate groups. These include the lack of an amphipathic N-terminal ‘elbow helix’ and no extracellular reentrant helices between TM5 and TM6. In addition, MacB contains only four proper TM helices as well as an additional coupling helix, thereby defining a separate transporter architecture. In accordance with differences in TMD topologies, the LptFG and MacB transporters also display diverging dimerization interfaces. Thus, we have chosen to assign LptFG and MacB to separate types. This notion is corroborated by the TM-score-based quantitative analysis (Table S6 and Fig. S1). Of note, at the time of writing, publicly available, yet unpublished structures of the lipid transporter complex MlaFEDB of *Gram*-negative bacteria reveal some resemblance of MlaE to LptF/G and MacB. However, the number of TM helices differs between LptFG (six TM helices), MlaE (five TM helices), and MacB (four TM helices) [45–48] (Table S6 and Fig. S1).

We would like to point out that the classification of the mammalian ABC transporters into the ABCA-G subfamilies can be maintained as subcategories of type IV (subfamilies B–D) and type V (subfamilies A and G) within the new nomenclature (Table 2). We are also not proposing any changes to gene symbols. Most importantly, the new nomenclature based on TMD architecture can be universally applied to ABC transporters beyond their particular physiological functions and across the three domains of life. Hence, it allows any newly discovered transporter fold to be compared with the existing types and seamlessly incorporated into the classification scheme, possibly as a new type. Since the new nomenclature depends on TMD architecture, it requires structural information in order to classify new transporter systems. At the same time, we regard the nomenclature as a dynamic platform that can be upgraded, adjusted, or refined whenever necessary due to novel insights that add extra dimensions to our understanding of ABC systems.

The recent advances in structural mapping of the diverse superfamily of ABC transporters have revealed a vast area of mechanistically uncharted territory. One key objective of future research should be to fully comprehend how type IV systems perform so many different functions, i.e., as importer, exporter, lipid floppase, ion channel, and regulator, by employing a single structural scaffold. However, we do not exclude that other types might turn out to be as functionally diverse as type IV systems. Exploring the different modes of operation and accompanying conformational landscapes [49] and the dynamics of the multifarious ABC systems will require integrative experimental

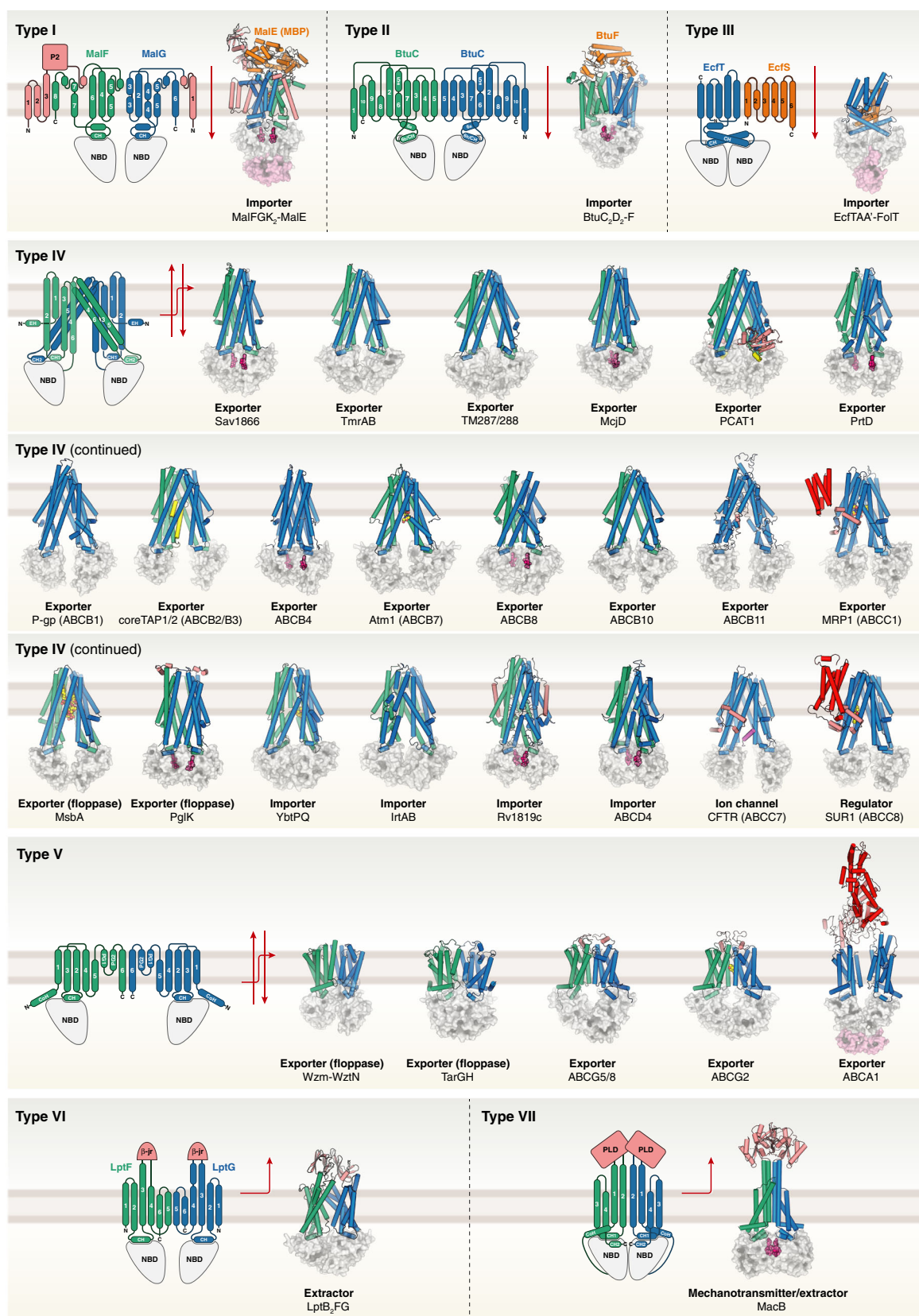


Fig. 1. The different types within the ABC transporter superfamily. Members of the superfamily of ABC transporters can be grouped into distinct types based on their TMD fold. The TMDs of representative experimentally determined structures are depicted as cartoons, and their NBDs are shown in surface representation. The TMD architecture of the first structure of each type is illustrated by a topology diagram. The number of structures shown for each transporter type does not necessarily reflect the abundance or importance of the respective type, but highlights the common scaffold and functional diversity of the transporters. The two TMDs of each transporter are shown in green and blue, respectively, except for cases where the TMDs are part of the same polypeptide chain (uniform blue color). Please note that the type V ABC transporters also include the retina-specific importer ABCA4 and importers in plants. Substrate-binding components of type I-III folds are illustrated in orange, and auxiliary domains and additional (TM) helices are shown in red, salmon, and violet, respectively. Bound (occluded) nucleotides and Mg^{2+} ions in the NBDs are shown as dark pink spheres. Transported substrates and inhibitors are shown in yellow (carbon) and in CPK colors (remaining atoms in small-molecule compounds), respectively. The directions of substrate transport are indicated by solid and dashed red arrows. The structures have the following Protein Data Bank (PDB) accession codes: MalFGK₂-MalE: [2R6G](#) [12]; BtuC₂D₂-BtuF: [4FI3](#) [50]; EcfTAA'-FolT: [4HUQ](#) [14]; Sav1866: [2HYD](#) [15]; TmrAB: [5MKK](#) [51]; TM287/288: [4Q4H](#) [52]; McjD: [4PLO](#) [53]; PCAT1: [6V9Z](#) [54]; Atm1: [4MYH](#) [55]; MRP1: [5UJA](#) [56]; PrtD: [5L22](#) [57]; P-gp: [4M1M](#) [58]; TAP1/2: [5U1D](#) [59]; ABCB4: [6S7P](#) [60]; ABCB8: [5OCH](#); ABCB10: [3ZDQ](#) [61]; ABCB11: [6LR0](#) [62]; MsbA: [5TV4](#) [63]; PglK: [6HRC](#) [64]; YbtPQ: [6P6J](#) [31]; IrtAB: [6TEJ](#) [32]; Rv1819c: [6TQF](#) [33]; ABCD4: [6BJJ](#) [30]; CFTR: [5UAK](#) [65]; SUR1: [6BAA](#) [66]; Wzm-WztN: [6OIH](#) [25]; TarGH: [6JBH](#) [26]; ABCG5/8: [5DO7](#) [16]; ABCG2: [6HCO](#) [67]; ABCA1: [5XJY](#) [23]; LptB₂FG: [5X5Y](#) [17]; MacB: [5LJ7](#) [21]. ABC, ATP-binding cassette; β-jr, β-jellyroll-like domain; C, C terminus; CH, coupling helix; CoH, connecting helix; EH, elbow helix; N, N terminus; NBD, nucleotide-binding domain; P2, extracytoplasmic loop; PG, periplasmic gate helix; PLD, periplasmic domain; TMD, transmembrane domain.

Table 1. Prokaryotic ABC transporters classified according to their TMD folds.

TMD fold	TM helix organization	Experimentally determined structures	PDB codes ^a	Function
Type I	(5-6) + (5-6/8) ^b	MalFGK ₂ (-E)	2R6G , 3FH6 , 3PUV , 3PUW , 3PUX , 3RLF , 4JBW	Maltose import
		ModB ₂ C ₂ (-A)	2ONK , 3D31	Molybdate import
		MetNI(-Q)	3DHW , 3TUI , 3TUJ , 3TUZ , 6CVL	Methionine import
		Art(QN) ₂	4YMS , 4YMT , 4YMU , 4YMV , 4YMW	Amino acid import
		AlgM1M2SS-Q2	4TQU	Alginate import
Type II	10 + 10	BtuC ₂ D ₂ (-F)	1L7V , 2QI9 , 4DBL , 4FI3 , 4R9U	Cobalamin import
		MolBC	2NQ2	Import of molybdate and tungstate
		HmuUV	4G1U	Heme import
		BhuUV(-T)	5B57 , 5B58	Heme import
Type III	4-8 (T) + 6-7 (S)	EcfTAA'-FolT	4HUQ , 5D3M , 5JSZ	Folate import
		EcfTAA'-PdxU2	4HZU	Pyridoxine import
		LbECF-PanT	4RFS	Pantothenate import
		CbiMQO	5X3X , 5X41	Co ²⁺ import
		ECF-CbrT	6FNP	Cobalamin import
Type IV	6 + 6	Sav1866	2HYD , 2ONJ	Multidrug export
	Homodimer	MsbA	3B60 , 3B5Y , 3B5Z , 5TV4 , 6BPL , 6BPP , 6BL6 , 6O30 , 6UZ2 , 6UZL	Lipid A/LPS flopping
	Heterodimer			
	Single chain	NaAtm1	4MRR , 4MRS , 4MRV , 4MRN , 4MRP	Export of GSH, GSH-related compounds, and metal-GSH complexes
		TM287/288	4Q4A , 4Q4H , 4Q4J , 6QUZ , 6QV0 , 6QV1 , 6QV2	Daunorubicin export
		McjD	4PLO , 5EG1 , 5OFR	Antimicrobial peptide export
		PCAT1	4RY2 , 6V9Z	Polypeptide export
		PglK	5C76 , 5C78 , 5NBD , 6HRC	Export (flopping) of lipid-linked oligosaccharides
		TmrAB	5MKK , 6RAF , 6RAG , 6RAH , 6RAI , 6RAJ , 6RAK , 6RAL , 6RAM , 6RAN	Peptide export
		PrtD	5L22	Polypeptide type-1 secretion system
		YbtPQ	6P6I , 6P6J	Metal-siderophore import
		Rv1819c	6TQE , 6TQF	Import of cobalamin and bleomycin
		IrtAB	6TEJ	Iron-siderophore import

Table 1. (Continued).

TMD fold	TM helix organization	Experimentally determined structures	PDB codes ^a	Function
Type V	6 + 6 Homodimer Heterodimer Single chain	Wzm-WztN TarGH	6OIH , 6M96 6JBH	O-antigen export (flopping) Export (flopping) of wall teichoic acid
Type VI	6 + 6 Heterodimer	LptB ₂ FG(C)	5X5Y , 5L75 , 6MIT , 6MJP , 6MHU , 6MHZ , 6MI7 , 6MI8 , 6S8G , 6S8H , 6S8N	LPS extraction
Type VII	4 + 4	MacB	5GKO , 5WS4 , 5LIL , 5LJ6 , 5LJ7 , 5XU1	Export of macrolides and polypeptide virulence factors

GSH, glutathione; LPS, lipopolysaccharide.

^aOnly PDB codes of structures with an overall resolution equal to or better than 4.5 Å were included.; ^bConserved TMs in bold.**Table 2.** Eukaryotic ABC transporters classified according to their TMD folds^a.

TMD fold	TM helix organization	Experimentally determined structures	PDB codes ^b	Function
Type IV	6 + 6 Homodimer Heterodimer Single chain	ABCB subfamily P-gp (ABCB1) <i>CmABCB1</i> <i>ScAtm1</i> (ABCB7) TAP1/2 (ABCB2/3) ABCB4 ABCB8 ABCB10 ABCB11 ABCC subfamily MRP1 (ABCC1) CFTR (ABCC7) SUR1 (ABCC8)	4F4C , 4M1M , 4M2S , 4M2T , 4Q9H , 4Q9I , 4Q9J , 4Q9K , 4Q9L , 4XWK , 5KPD , 5KPI , 5KPJ , 5KO2 , 5KOY , 6C0V 3WME , 3WMF , 3WVG , 6A6M , 6A6N 4MYC , 4MYH 5U1D 6S7P 5OCH 3ZDQ , 4AYT , 4AYW , 4AYX 6LR0 5UJA , 5UJ9 , 6BHU , 6UY0 5UAR , 5UAK , 5W81 , 6D3R , 6MSM , 6O1V , 6O2P 6BAA , 6C3O , 5YKE , 5YKF , 5YWC , 5YWD , 5YW7 , 5YW8 , 6JB1 , 6JB3 , 6PZ9 , 6PZA , 6PZC , 6PZI	Multidrug export Multidrug export Unknown substrate for Fe/S protein biogenesis Peptide export Lipid export Unknown Unknown Bile salt export Leukotriene, sphingolipid, and multidrug export Chloride channel Regulatory module of K _{ATP} channel
Type V	6 + 6 Homodimer Heterodimer Single chain	ABCD subfamily ABCD4 ABCA subfamily ABCA1 ABCG subfamily ABCG5/8 ABCG2	6JBJ 5XJY 5DO7 5NJG , 5NJ3 , 6ETI , 6FEQ , 6FFC , 6HIJ , 6HCO , 6HBU , 6HZM , 6VXF , 6VXH , 6VXI , 6VXJ	Cobalamin import Phospholipid/cholesterol export Sterol export Multidrug export

^aExcluding ABC proteins of the ABCH and ABCI subfamilies, which most likely can be classified as type V and type III systems, respectively.; ^bOnly PDB codes of structures with an overall resolution equal to or better than 4.5 Å were included.

approaches that include electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), single-molecule techniques, and single-turnover

experiments. We are confident that future studies of such kind will provide major new insights into the mechanisms of these fascinating molecular machines.

Acknowledgements

K.B. acknowledges support by a grant of the Medical Research Council (MR/N020103/1). M.D. is supported in part by the Intramural Program of the NIH. V.K. acknowledges support by the Medical Research Council (MR/N000994/1) and Wellcome Trust (101828/Z/13/Z). R.L. acknowledges generous financial support from German Research Foundation (LI 415/5). D.P.T. is supported in part by the Canada Research Chairs program. This work was supported by the German Research Foundation (SFB 807 and TA157/12-1 (Reinhart Koselleck Award Program) to R.T.).

Author contributions

CT and RT wrote the manuscript with contributions from all coauthors. This review is the quintessence of a resumed discussion that started at the FEBS Advanced Lecture Course on the Biochemistry of Membrane Proteins in Budapest (2019) and continued at the FEBS Conference on ATP-Binding Cassette (ABC) Proteins in Innsbruck (2020). The discussion included a vivid exchange of thoughts *via* hundreds of emails and remote video sessions during the global COVID-19 pandemic. In addition to the authors listed, we received positive feedbacks on our proposed classification from several further leading scientists in the ABC transporter field. Yet, as they felt that their contribution was too small, they decided not to accept authorship.

References

- Higgins CF, Hiles ID, Salmond GPC, Gill DR, Downie JA, Evans IJ, Holland IB, Gray L, Buckel SD, Bell AW *et al.* (1986) A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria. *Nature* **323**, 448–450.
- Dassa E and Bouige P (2001) The ABC of ABCs: a phylogenetic and functional classification of ABC systems in living organisms. *Res Microbiol* **152**, 211–229.
- Bouige P, Laurent D, Piloyan L and Dassa E (2002) Phylogenetic and functional classification of ATP-binding cassette (ABC) systems. *Curr Protein Pept Sci* **3**, 541–559.
- Saurin W, Hofnung M and Dassa E (1999) Getting in or out: early segregation between importers and exporters in the evolution of ATP-binding cassette (ABC) transporters. *J Mol Evol* **48**, 22–41.
- Dean M, Rzhetsky A and Allikmets R (2001) The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* **11**, 1156–1166.
- Klein I, Sarkadi B and Varadi A (1999) An inventory of the human ABC proteins. *Biochim Biophys Acta* **1461**, 237–262.
- Tusnady GE, Sarkadi B, Simon I and Varadi A (2006) Membrane topology of human ABC proteins. *FEBS Lett* **580**, 1017–1022.
- Gerovac M and Tampé R (2019) Control of mRNA translation by versatile ATP-driven machines. *Trends Biochem Sci* **44**, 167–180.
- Khawaja M, Ma Q and Saier MH Jr (2005) Topological analysis of integral membrane constituents of prokaryotic ABC efflux systems. *Res Microbiol* **156**, 270–277.
- Wang B, Dukarevich M, Sun EI, Yen MR and Saier MH Jr (2009) Membrane porters of ATP-binding cassette transport systems are polyphyletic. *J Membr Biol* **231**, 1–10.
- Locher KP, Lee AT and Rees DC (2002) The E. coli BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science* **296**, 1091–1098.
- Oldham ML, Khare D, Quirocho FA, Davidson AL and Chen J (2007) Crystal structure of a catalytic intermediate of the maltose transporter. *Nature* **450**, 515–521.
- Wang T, Fu G, Pan X, Wu J, Gong X, Wang J and Shi Y (2013) Structure of a bacterial energy-coupling factor transporter. *Nature* **497**, 272–276.
- Xu K, Zhang M, Zhao Q, Yu F, Guo H, Wang C, He F, Ding J and Zhang P (2013) Crystal structure of a folate energy-coupling factor transporter from *Lactobacillus brevis*. *Nature* **497**, 268–271.
- Dawson RJ and Locher KP (2006) Structure of a bacterial multidrug ABC transporter. *Nature* **443**, 180–185.
- Lee J-Y, Kinch LN, Borek DM, Wang J, Wang J, Urbatsch IL, Xie X-S, Grishin NV, Cohen JC, Otwinowski Z *et al.* (2016) Crystal structure of the human sterol transporter ABCG5/ABCG8. *Nature* **533**, 561–564.
- Luo Q, Yang X, Yu S, Shi H, Wang K, Xiao L, Zhu G, Sun C, Li T, Li D *et al.* (2017) Structural basis for lipopolysaccharide extraction by ABC transporter LptB2FG. *Nat Struct Mol Biol* **24**, 469–474.
- Dong H, Zhang Z, Tang X, Paterson NG and Dong C (2017) Structural and functional insights into the lipopolysaccharide ABC transporter LptB2FG. *Nat Commun* **8**, 222.
- Fitzpatrick AWP, Llabrés S, Neuberger A, Blaza JN, Bai X-C, Okada U, Murakami S, van Veen HW, Zachariae U, Scheres SHW *et al.* (2017) Structure of the MacAB-TolC ABC-type tripartite multidrug efflux pump. *Nat Microbiol* **2**, 17070.
- Okada U, Yamashita E, Neuberger A, Morimoto M, van Veen HW and Murakami S (2017) Crystal structure of tripartite-type ABC transporter MacB from *Acinetobacter baumannii*. *Nat Commun* **8**, 1336.

- 21 Crow A, Greene NP, Kaplan E and Koronakis V (2017) Structure and mechanotransmission mechanism of the MacB ABC transporter superfamily. *Proc Natl Acad Sci USA* **114**, 12572–12577.
- 22 Yang HB, Hou WT, Cheng MT, Jiang YL, Chen Y and Zhou CZ (2018) Structure of a MacAB-like efflux pump from *Streptococcus pneumoniae*. *Nat Commun* **9**, 196.
- 23 Qian H, Zhao X, Cao P, Lei J, Yan N and Gong X (2017) Structure of the human lipid exporter ABCA1. *Cell* **169**, 1228–1239.e10.
- 24 Taylor NMI, Manolaridis I, Jackson SM, Kowal J, Stahlberg H and Locher KP (2017) Structure of the human multidrug transporter ABCG2. *Nature* **546**, 504–509.
- 25 Bi Y, Mann E, Whitfield C and Zimmer J (2018) Architecture of a channel-forming O-antigen polysaccharide ABC transporter. *Nature* **553**, 361–365.
- 26 Chen L, Hou W-T, Fan T, Liu B, Pan T, Li Y-H, Jiang Y-L, Wen W, Chen Z-P, Sun L *et al.* (2020) Cryo-electron microscopy structure and transport mechanism of a wall teichoic acid ABC transporter. *MBio* **11**, e02749–19.
- 27 Koch J, Guntrum R, Heintke S, Kyritsis C and Tampé R (2004) Functional dissection of the transmembrane domains of the transporter associated with antigen processing (TAP). *J Biol Chem* **279**, 10142–10147.
- 28 Thomas C and Tampé R (2020) Structural and mechanistic principles of ABC transporters. *Annu Rev Biochem* **89**, 605–636.
- 29 Scheepers GH, Lycklama ANJA and Poolman B (2016) An updated structural classification of substrate-binding proteins. *FEBS Lett* **590**, 4393–4401.
- 30 Xu D, Feng Z, Hou WT, Jiang YL, Wang L, Sun L, Zhou CZ and Chen Y (2019) Cryo-EM structure of human lysosomal cobalamin exporter ABCD4. *Cell Res* **29**, 1039–1041.
- 31 Wang Z, Hu W and Zheng H (2020) Pathogenic siderophore ABC importer YbtPQ adopts a surprising fold of exporter. *Sci Adv* **6**, eaay7997.
- 32 Arnold FM, Weber MS, Gonda I, Gallenito MJ, Adenau S, Egloff P, Zimmermann I, Hutter CAJ, Hürlimann LM, Peters EE *et al.* (2020) The ABC exporter IrtAB imports and reduces mycobacterial siderophores. *Nature* **580**, 413–417.
- 33 Rempel S, Gati C, Nijland M, Thangaratnarajah C, Karyolaimos A, de Gier JW, Guskov A and Slotboom DJ (2020) A mycobacterial ABC transporter mediates the uptake of hydrophilic compounds. *Nature* **580**, 409–412.
- 34 Shitan N, Bazin I, Dan K, Obata K, Kigawa K, Ueda K, Sato F, Forestier C and Yazaki K (2003) Involvement of CjMDR1, a plant multidrug-resistance-type ATP-binding cassette protein, in alkaloid transport in *Coptis japonica*. *Proc Natl Acad Sci USA* **100**, 751–756.
- 35 Terasaka K, Blakeslee JJ, Titapiwatanakun B, Peer WA, Bandyopadhyay A, Makam SN, Lee OR, Richards EL, Murphy AS, Sato F *et al.* (2005) PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. *Plant Cell* **17**, 2922–2939.
- 36 Lee M, Choi Y, Burla B, Kim Y-Y, Jeon B, Maeshima M, Yoo J-Y, Martinoia E and Lee Y (2008) The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO₂. *Nat Cell Biol* **10**, 1217–1223.
- 37 Yang H and Murphy AS (2009) Functional expression and characterization of Arabidopsis ABCB, AUX 1 and PIN auxin transporters in *Schizosaccharomyces pombe*. *Plant J* **59**, 179–191.
- 38 Kamimoto Y, Terasaka K, Hamamoto M, Takanashi K, Fukuda S, Shitan N, Sugiyama A, Suzuki H, Shibata D, Wang B *et al.* (2012) Arabidopsis ABCB21 is a facultative auxin importer/exporter regulated by cytoplasmic auxin concentration. *Plant Cell Physiol* **53**, 2090–2100.
- 39 Shitan N, Dalmas F, Dan K, Kato N, Ueda K, Sato F, Forestier C and Yazaki K (2013) Characterization of *Coptis japonica* CjABCB2, an ATP-binding cassette protein involved in alkaloid transport. *Phytochemistry* **91**, 109–116.
- 40 Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A *et al.* (1997) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* **277**, 1805–1807.
- 41 Quazi F, Lenevich S and Molday RS (2012) ABCA4 is an N-retinylidene-phosphatidylethanolamine and phosphatidylethanolamine importer. *Nat Commun* **3**, 925.
- 42 Kang J, Hwang JU, Lee M, Kim YY, Assmann SM, Martinoia E and Lee Y (2010) PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proc Natl Acad Sci USA* **107**, 2355–2360.
- 43 Xi J, Xu P and Xiang CB (2012) Loss of AtPDR11, a plasma membrane-localized ABC transporter, confers paraquat tolerance in *Arabidopsis thaliana*. *Plant J* **69**, 782–791.
- 44 Kang J, Yim S, Choi H, Kim A, Lee KP, Lopez-Molina L, Martinoia E and Lee Y (2015) Abscisic acid transporters cooperate to control seed germination. *Nat Commun* **6**, 8113.
- 45 Coudray N, Isom GL, MacRae MR, Saiduddin MN, Bhabha G and Ekiert DC (2020) Structure of MlaFEDB lipid transporter reveals an ABC exporter fold and two bound phospholipids. *bioRxiv* <https://doi.org/10.1101/2020.06.02.129247>
- 46 Mann D, Fan J, Farrell DP, Somboon K, Andrew Muenks S, Tzokov S, Khalid F, Dimaio SM and Bergeron JRC (2020) Structural basis for lipid transport by the MLA complex. *bioRxiv* <https://doi.org/10.1101/2020.05.30.125013>

- 47 Tang X, Chang S, Qiao W, Luo Q, Chen Y, Jia Z, Coleman J, Zhang K, Wang T, Zhang Z *et al.* (2020) Structural insight into outer membrane asymmetry maintenance of Gram-negative bacteria by the phospholipid transporter MlaFEDB. *bioRxiv* <https://doi.org/10.1101/2020.06.04.133611>
- 48 Chi X, Fan Q, Zhang Y, Liang K, Wan L, Zhou Q and Li Y (2020) Structural mechanism of phospholipids translocation by MlaFEDB complex. *Cell Res.* <https://doi.org/10.1038/s41422-020-00404-6>
- 49 Hofmann S, Janulienė D, Mehdipour AR, Thomas C, Stefan E, Bruchert S, Kuhn BT, Geertsma ER, Hummer G, Tampé R *et al.* (2019) Conformation space of a heterodimeric ABC exporter under turnover conditions. *Nature* **571**, 580–583.
- 50 Korkhov VM, Mireku SA and Locher KP (2012) Structure of AMP-PNP-bound vitamin B12 transporter BtuCD-F. *Nature* **490**, 367–372.
- 51 Nöll A, Thomas C, Herbring V, Zollmann T, Barth K, Mehdipour AR, Tomasiak TM, Bruchert S, Joseph B, Abele R *et al.* (2017) Crystal structure and mechanistic basis of a functional homolog of the antigen transporter TAP. *Proc Natl Acad Sci USA* **114**, E438–E447.
- 52 Hohl M, Hurlimann LM, Böhm S, Schoppe J, Grutter MG, Bordignon E and Seeger MA (2014) Structural basis for allosteric cross-talk between the asymmetric nucleotide binding sites of a heterodimeric ABC exporter. *Proc Natl Acad Sci USA* **111**, 11025–11030.
- 53 Choudhury HG, Tong Z, Mathavan I, Li Y, Iwata S, Zirah S, Rebuffat S, van Veen HW and Beis K (2014) Structure of an antibacterial peptide ATP-binding cassette transporter in a novel outward occluded state. *Proc Natl Acad Sci USA* **111**, 9145–9150.
- 54 Kieuvongngam V, Olinares PDB, Palillo A, Oldham ML, Chait BT and Chen J (2020) Structural basis of substrate recognition by a polypeptide processing and secretion transporter. *Elife* **9**, e51492.
- 55 Srinivasan V, Pierik AJ and Lill R (2014) Crystal structures of nucleotide-free and glutathione-bound mitochondrial ABC transporter Atm1. *Science* **343**, 1137–1140.
- 56 Johnson ZL and Chen J (2017) Structural basis of substrate recognition by the multidrug resistance protein MRP1. *Cell* **168**, 1075–1085.e9.
- 57 Morgan JLW, Acheson JF and Zimmer J (2017) Structure of a type-1 secretion system ABC transporter. *Structure* **25**, 522–529.
- 58 Li J, Jaimes KF and Aller SG (2014) Refined structures of mouse P-glycoprotein. *Protein Sci* **23**, 34–46.
- 59 Oldham ML, Grigorieff N and Chen J (2016) Structure of the transporter associated with antigen processing trapped by herpes simplex virus. *eLife* **5**, e21829.
- 60 Olsen JA, Alam A, Kowal J, Stieger B and Locher KP (2020) Structure of the human lipid exporter ABCB4 in a lipid environment. *Nat Struct Mol Biol* **27**, 62–70.
- 61 Shintre CA, Pike ACW, Li Q, Kim J-I, Barr AJ, Goubin S, Shrestha L, Yang J, Berridge G, Ross J *et al.* (2013) Structures of ABCB10, a human ATP-binding cassette transporter in apo- and nucleotide-bound states. *Proc Natl Acad Sci USA* **110**, 9710–9715.
- 62 Wang L, Hou WT, Chen L, Jiang YL, Xu D, Sun L, Zhou CZ and Chen Y (2020) Cryo-EM structure of human bile salts exporter ABCB11. *Cell Res* **30**, 623–625.
- 63 Mi W, Li Y, Yoon SH, Ernst RK, Walz T and Liao M (2017) Structural basis of MsaA-mediated lipopolysaccharide transport. *Nature* **549**, 233–237.
- 64 Perez C, Mehdipour AR, Hummer G and Locher KP (2019) Structure of outward-facing PglK and molecular dynamics of lipid-linked oligosaccharide recognition and translocation. *Structure* **27**, 669–678.e5.
- 65 Liu F, Zhang Z, Csanady L, Gadsby DC and Chen J (2017) Molecular structure of the human CFTR ion channel. *Cell* **169**, 85–95.e8.
- 66 Martin GM, Yoshioka C, Rex EA, Fay JF, Xie Q, Whorton MR, Chen JZ and Shyng SL (2017) Cryo-EM structure of the ATP-sensitive potassium channel illuminates mechanisms of assembly and gating. *Elife* **6**, e24149.
- 67 Manolaridis I, Jackson SM, Taylor NMI, Kowal J, Stahlberg H and Locher KP (2018) Cryo-EM structures of a human ABCG2 mutant trapped in ATP-bound and substrate-bound states. *Nature* **563**, 426–430.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Phylogenetic tree based on TM-scores of structural TMD alignments.

Table S1. TM-scores based on pairwise structural alignment of representatives of the different TMD types.

Table S2. TM-scores based on pairwise structural alignment of type I TMDs.

Table S3. TM-scores based on pairwise structural alignment of type II TMDs.

Table S4. TM-scores based on pairwise structural alignment of type IV TMDs in inward-facing conformations.

Table S5. TM-scores based on pairwise structural alignment of type IV TMDs in (semi-) occluded/outward-facing conformations.

Table S6. TM-scores based on pairwise structural alignment of type V, VI, and VII TMDs^a.